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THE HARDINESS OF FISH AND INVERTEBRATES UNDER CHANGES IN THE SALINITY OF THEIR ENVIRONMENT AND THE METHODS OF THE DETERMINATION OF THIS HARDINESS

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The Hardiness of Fish and Invertebrates Under Changes in the Salinity of Their Environment And the Methods of the Determination of this Hardiness

Vynoslivost' ryb i bespozvonocnyx pri izmenenii solenosti sredy i metodiki ee opredelenija

by

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In connection with the broadening of work on the reconstruction of the fauna of oceanic water basins and salt-water lakes, interest towards the determination of the demands made by industrial and food types of water animals on the salinity of the environment has strengthened. Investigations of this sort have become part of the plans of various organizations. Many of the works that have been begun have already

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been published (Gosteeva, 1954; Iriximovič, 1951; Karpevič, 1946, 1947, 1953, 1955, and others; Kirpičnikov, 1957; Konovalov, 1950; Leščinskaja, 1955; Logovinovič, 1955; Nikitin and Turpaeva, 1953, and others).

From the ennumeration of articles adduced above it is apparent to what a degree researches on the given question are being carried out. This is understandable enough, since the regulation of the out-flow of southern rivers causes considerable changes in the physico-chemical and saline regimes of the seas.

As is well known, any change in the surrounding environment influences the composition, numbers, or distribution of species, and, therefore, in order to clarify the degree of this influence, one must determine the sensativity of separate species to the effect of environmental factors and their combinations.

In the past few years, more and more importance has been attached to works on the acclimatization of oceanic and salt-water organisms. In view of the fact that almost all of the salt-water water basins of the USSR (seas and lakes) have their individual characteristics, one of the most important questions during the solution of biologically founded acclimatization methods is the clarification of the relationship of transplanted subjects both to general salinity, and to the saline composition of their new environment.

The methods described in the present article are intended for biologists studying the ecologo-physical characteristics of water organisms in semi-field, temporary, and stationary ecologo-physiological laboratories.

Salinity Tolerance: the Types and the Determining Factors.

The seas which wash the shores of our country, their bays and estuaries, all are distinguished one from another both by the general salt content, as well as by their ionnic make-up (Table 1). For example, the composition of salts in the Baltic, Black, Azov, and White Seas is close to that of the ocean, but, in respect to the number of salts dissolved in one liter, they differ rather strongly. The general average salinity of the surface layers of the waters of the White Sea is equal to 25-26%, while the bottom layers are equal to 31%; for the Black Sea the correspondance is about 18% and 22%.

The waters of other groups of seas--the Azovian, Caspian, and Aral Seas--are close one to another in regard to the general quantity of salts dissolved in one liter. The average salinity of their waters is close, and varies within the limits of 11-13%, but the ion content of the salts in each sea has its individual traits. For example, the relative content of two valent ions--magnesium, calcium,

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Table 1

The Salt Content of the Waters of the Ocean and Saline Water Basins In % by Weight

1 Ионы	2 Океан (по Дитмару)	3 Моря				4 Озеро Балхаш (по Бурмакину и Домрачеву, в % экв.)	
		a Балтийское (по Заринс и Озолиной)	b Азовское (по Мусиной и Микей)	c Каспийское (по Бруевичу)	d Аральское (по Блинову)	Западный	Восточный
Na^{+}	30,59	30,10	30,51	24,81	21,94	33,4	36,51
K^{+}	1,11	1,08	0,89	0,66	1,15	Следы	2,96
Ca^{++}	1,20	1,44	1,43	2,70	4,65	11,9	0,9
Mg^{++}	3,75	3,8	3,7	5,70	5,18	4,7	12,6
Cl^{-}	55,29	54,85	54,65	41,78	34,44	8,7	20,7
SO_4^{--}	7,69	7,7	8,08	23,49	31,07	25,0	16,0
CO_3^{--}	0,21	—	0,55	—	1,57	—	3,7
HCO_3^{-}	—	1,3	—	0,86	—	16,3	9,6
5 Соленость в ‰	34,3	6,6	11,8	12,66	10,6	0,4—0,8	4,6

Key: 1-Ions
2-The ocean (according to Dietmar)
3-Seas
a) the Baltic (according to Zarins and Ozolina)
b) the Sea of Azov (according to Musina and Mikej)
c) the Caspian (according to Bruevic)
d) the Aral (according to Blinov)
4-the Lake Balxash (according to Burmakin and Domracev)
a) Western
b) Eastern
5-Salinity in %

and the sulphonic-acid group (Mg", Ca", and SO_4")--increases in the Caspian and Aral Seas in comparison with the ocean. The content of monovalent ions, which are extremely active in the preservation of osmotic pressure in aquatic organisms, falls, however.

The curves for the freezing points of waters with differing salinity from the Aral Sea, the Caspian Sea, and the Sea of Azov are also divergent.

A completely idiosyncratic content of salts has formed in certain lakes (Balkhash, Issyk-Kul', and others). In the first water basin the ions K', Na', and Mg" predominate (Table 1). However, the concentration of calcium during the increase in the concentration of salts in the waters of Balkhash is sharply diminished. The salt content of the waters of many other salt water lakes may have their special traits, and this is a fact which is obligatory to consider in ecological investigations.

The non-uniformity of the salt content causes peculiar response reactions in organisms settled in these waters to changes in the salinity of the environment. As far back as V. N. Beklemišev and V. P. Baskina (1933) it was established that the water of the Aral Sea was less harmful for freshwater species than ocean water, since bi-valent ions are

antagonistic to mono-valent ions, and soften their action. At the same time, the waters of the Aral and Caspian Seas may turn out to be not too hospitable for oceanic species, while the waters of Lake Balkhash, with a salinity higher than 2-3%, are hostile to the life of many invertebrates. In consequence of this fact, during the introduction of new species into these water basins it is necessary to have previously determined the action of their natural waters on aboriginal and transplanted species. Moreover, separate species of aquatic animals and plants react differently to changes in the salinity even of waters to which they are adapted. The salinity tolerance of industrial and food species depends most of all on the following factors.

1. On the origin of the species. Fresh-water forms, as a rule, are more stenohaline , for example, the fresh-water molluscs (Anodonta piscinalis and others) may live in the Sea of Azov with a salinity not higher than 2%. Molluscs of salt-water origin (Dreissena polymorpha, Monodacna colorata) are more eugenohaline ; they withstand exposure to Azovian water with a salinity of from 0 to 5-7%. Molluscs of oceanic origin (Cardium edule, Syndesmia ovata, and others) are even more eugenohaline : their salinity range is from 5-7 up to 4% (Karpevič, 1952, and elsewhere).

2. On the level of organization of the species, and on the structure of the osmosis regulatory apparatus of the individual. For example, the
of species of fresh water origin which have achieved different steps on the evolutionary ladder is different: edentates survive Azov water with a salinity of 0-2%, Limnodritus with a salinity of 0-5%, and the cray-fish Acanthocyclops vernalis with a salinity of from 0 to 10-12%.

3. The saline tolerance of a species depends on the stage of development of its individuals. For example, adult Corbulomia maeotica in an adult stage survive in Azov water with a salinity of 7.5-17-20%, while the development of eggs is most satisfactory within a range of from ten to fifteen percent (Karpevič, 1955). Adult individuals of Monodacna colorata from the Taganrog Estuary live in fresh, as well as slightly saline water (from 0-7-9%), while their larvae may form a shell (according to L. P. Maksimova) only in Azov water with a salinity not lower than 0.5-1%. The young of the bream survive well in the waters of the Don and of the Sea of Azov with a salinity of 6-7%, yearlings up to 10%, and adults up to 11-11.5%. Moreover, the shells of the eggs of several species of fish (bullheads and many others) are impervious to salt, and, therefore, the development of the embrio flows normally under almost any degree of salinity (5-30%), but the hatched young in water with high salinity

quickly loose tissue water, which destroys their conversion of energy, and die within twenty-four hours.

4. Salinity tolarance in an organism depends, as well, on the inter-relationships of the ion content of the salts of the surrounding environment. For example, the widespread mollusc Cardium edule, under a sharp change in salinity, lives in Azov water, with a temperature of 20-24°, in a range of 5-25‰ (2.7-12.3‰ Cl'), in Caspian water (6-16°) in a range of 7-31‰ (2.9-13‰ Cl'), and in Aral water (6-16°) in a range of 6-8 to 40‰ (from 2.1-2.8 to 14‰ Cl').[1] Thus, the salinity range of Cardium edule, determined by the indicators of general salinity, is smallest in Azov water, while in Aral water Cardium edule is most
If one turns attention to the chlorine content, then one may mention that in all cases the range of survival for Cardium edule is almost identical; the low limit of survival is limited by a chlorine content of about 2.8‰, and the high

[1] A more exact determination of the salinity range of a species in relation to the chlorine of the water demands more exact data on the relation of the ions of salts in solution and on the homogeneity of temperature during experimentation.

limit by a content of 12.3-14%. We have observed the same thing both in Adacna and Dreissena. This phenomenon, which we have investigated in detail, depends entirely on the idiosyncracy of the ion content of the experimental waters (Karpevič, 1953).

5. The salt endurance of an individual depends on the speed of changes in salinity in the surrounding environment.

Dreissena from the river Volga (Dreissena polymorpha var. fluviatilis) accept an increase in the salinity of Caspian water only for a range of 5-6% (2.5% Cl'), if the increase is sudden, i.e., if the molluscs are at once thrown from fresh water into the Caspian water with a salinity of 6% (Karpevič, 1947, 1953). If, however, the salinity of the vessels, in which are the molluscs, is increased slowly (by 2% over 48 hours), considering the time necessary for the physiological adaptation of an individual to a changed salinity, then the salinity range for the very same molluscs is increased almost two times, and they live in water with a salinity higher than that mentioned above, i.e., in the range of 0-11% (up to 4.6% Cl'). Moreover, they can endure, in the course of two weeks, Caspian water with a salinity of 11-17%.

Of especial importance for invertebrates is physiological adaptation in cases where heavy, bi-valent ions predominate in sea water. These ions penetrate the organism only very

slowly, and, therefore, the process of the regulation of osmosis is slowed, or the regulation of osmosis during the first hours of change in the salinity of the environment is carried out mainly at the cost of water, and not salt, exchange. In consequence of this fact, during a sharp change in the salinity of Caspian or Aral waters the inhabitants sometimes die from a considerable and quick loss of water by the tissues, or from their flooding, and not from the inhospitible salinity.

6. The saline tolerance of an organism changes from the simultaneous action of several factors in the environment. For example, if the conditions necessary for the presence of a given species are completely favorable, and only a single one of these factors is sublethal, an individual in such conditions can survive a rather long time (weeks and months), but if the indicators of two or several environmental factors (temperature, salinity, oxygen content, etc.) are sublethal for a given species, their harmful effects are compounded and the death of the organism is hastened. For example, Dreissena andrusovi, under a temperature of 12-20°, survives well in a salinity range of 2-12‰ (Caspian water) and lives rather long in water with a sublethal salinity--fresh and Caspian (12-17‰), but, when the temperature is raised almost to critical levels (28°), the dreissena may

survive only in an hospitable salinity range (2-10‰), and die very quickly in sublethal ranges (Karpevič, 1947). We see the very same picture in the young of pike, bream, and other fish.

7. The saline tolerance of an individual of a species depends, as well, on the physiological organization. Many invertebrates and fish, especially adult individuals, can resist the action of sublethal salinity for a long time; this fact depends on their general physiological endurance, on the level of organization of their osmosis regulating organs, or on the degree of the insulation of their internal environment from the external environment, etc. This question has not yet been investigated.

The saline tolerance of a species may depend on even other causes, but the above mentioned reasons, in our opinion, are sufficient to allow one to imagine with what care one must approach the data of scattered or limited observations in nature. For instance, one can not accept as the saline range for a species the shortlived survival of separate individuals in extraordinary circumstances. For example, the worm Nereis in the northern Caspian is sometimes met with even in fresh water, a consequence of the extremely changing saline regime of this water basin. For several years the fresh water of the Volga has been washing over the habitats

of Nereis, which earlier had been under sea water. Thanks to physiological endurance they do not at once die in unfavorable circumstances, and are recovered live by bottom dragging apparatus. However, Nereis does not establish stabile populations in regions with such a changing saline environment (Karpevič, 1952). Without considering the mutability of the environment and the physiological endurance of individuals of separate species it is possible to draw mistaken conclusions.

In 1926, V. Ju. Marti (1938) observed the penetration of pike from the Sea of Azov to the Black Sea. Over a long period pike were caught along the shores of the Caucasus, and on this basis one may surmise that they are capable of surviving in water of Black Sea salinity (16-20‰). However, the pike did not entrench themselves in the new conditions because of a sharp worsening of their physiological condition, which appeared in darkening of the eyes, and so forth.

What we have said shows that, under a determined saline range for a species, it is necessary to carefully and thoughtfully evaluate the facts gathered in nature and by experiment. This is especially important for beginning investigators, all the more so since the methods used to research the saline tolerance of individuals are simple and easy to master. But there is no accurate description of

these methods, and various modifications appear which may lead to an extremely simplistic approach to an experiment, and may lead to the loss of precision and the incompatibility of results achieved by different authors. All these factors force us to indicate the difficulty in research in these regions, and to try to unify methods that are being used. Below we shall briefly outline the methods of determining the saline tolerance of species which are used in the ongoing and temporary ecologo-physiological laboratories of VNIRO.

The Method of Determining the Demands of Separate Species of Aquatic Animals on the Salinity of the Environment.

The Choice of Experimental Material.

Proceeding from the principle that species exist in the form of ecological groups (populations) and that each such group has adaptations characteristic of it, we recommend experimental material be taken from strictly determined areas, taking into account the conditions of the habitat of a given population. For example, Dreissena polymorpha from the rivers Čapaevka and the Volga have for generations lived in fresh water, and all their physiological processes have been adapted to fresh water and the fresh water type of osmosis regulation (Karpevič, 1955). In individuals of this

species taken from the weakly salted waters of the northern regions of the Caspian the basis of the physiological process is different, and the mixing of fresh water and salt-water types in experimentation may give inaccurate, unconvincing, or hard to understand results.

To achieve reliable data on the effect on an organism of salinity alone, and not some other factor of the environment, it is necessary that all other environmental factors under experimental conditions be in the optimal range for a given species. In containing animals one must also take into consideration their behaviour.

Certain individuals in an aquarium feel well only if they are in the presence of other animals like themselves (the herd drive). Khamsa and sardelle behave more quietly in a school with scattered light. Individual dreissena survive better in a druza attached to a substratum.[2] Other animals prefer isolation. Thus, Scorpaena porcus usually sits motionless in the corner of an aquarium, waiting for prey, but if they are confined in a close aquarium with other fish, they cease to feed. Bullheads also require

[2] A druza of molluscs is a group of thickly adhereing individuals, attached to a substratum.

dark places and hiding places (stones, vegetation, etc.). At spawning time male bullheads which have been placed in an aquarium with females tear off the fins of the latter, tear off their scales, and cause considerable wounds, from which the females quickly die. While being held in schools, adult individuals, especially males, eat faded individuals and the young, and, therefore, it is better to hold them in isolation.

Some animals in experimental conditions get along very easily without a foundation (molluscs, crabs, etc.), while others perish without such a foundation.

Taking into account the peculiarities of the behaviour of animals, one must all the same endevour to carry out experiments on mass material.

As a rule, experiments on the saline tolerance of a species should follow the developmental stages of an individual. But frequently, depending on the goals of an experiment, it is enough to determine the demands of only certain stages of development: only eggs, or spermatozoa, the larva or young, or half-grown individuals. Sometimes it is important to elucidate separately the reaction to salinity of males and females.

It is always necessary to separate the experimental material from outside bodies (from the mixture with different animals and plants, from the soil, and so forth), and before the

experiment to thoroughly count and weigh the individuals of each group separately, though sometimes weighing comes at the end of an experiment, due to the increased sensitivity of the experimental organisms.

The Preparation of Water with Varying Salinity.

For experiments we usually use natural sea or river water. The water is procured from open places in the sea or river in well washed, tightly closed, glass bottles. In the laboratory the water is kept in a dark and cool place. In the course of the first 10-20 days all organisms that have entered the bottle during filling have died, and all mineral and organic contaminants have settled to the bottom. At about this time the water becomes completely transparent and of good biological quality, i.e., suitable for the habitation of aquatic organisms; before its use one must carefully siphon off the upper layers into another vessel, not including the sediment at the bottom, and increase the oxygen content by stirring.

However, if large quantities of phyto- and zooplankton forms or mechanical suspensions are present (clay and sand particles, detritus, etc.) in the water captured by the bottle, then oxygen gradually dries out in it, and often hydrogen sulphide is formed. During this period which, as a rule, may last a week, the water is not suitable for

experimentation. Therefore, one must select the clearest sea or river water for the bottle, from places thinly inhabited by plankton. If not, one must carefully filter the water before its use or before long storage.

Long standing (over the course of years) of pure, transparent water in closed bottles in dark and cool places does not impair its quality, although it must be aerated before use.

Sea water with a higher degree of salinity than the natural water of a given basin is prepared by distillation in large, flat cuvettes under the rays of the sun. It is desirable to keep the cuvettes in open places, as high from the earth as possible, so that the irradiation by the sun be free, and that contamination with dust, etc., be avoided.

To distill water without destroying its natural ion content is possible only within certain limits. For example, Caspian water with a salinity of about 10‰ may, apparently, be distilled to 35-40‰, while Aral water of the same salinity may be distilled only up to 20-28‰ (Blinov, 1956). In the case of a large increase of the concentration of salts of Caspian and, especially, Aral water, calcium carbonate ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) precipitate on the

bottom of the vessel, which leads to the destruction of the inter-relationships in the ion content of the salts disolved in the water.

Concentration of natural Azov and Black Sea water with a salinity of about 10-18‰ without destroying its ion content is apparently possible within more important limits (up to 50-60‰). The limits indicated for the concentration of salts in sea waters, except for that of the Aral, are based on our observations, and still demand chemical verification.

In hyper-concentrated sea water the relative content of mono-valent ions is increased and the alkalinity changes; the action of such water on the experimental object may cause an unexpected reaction.

For this reason the distillation of the natural water of each basin is done separately, and only up to the critical concentration (the concentration at which the salt content of the water of a given basin starts to change), or, more often, up to the necessary concentration: 12.5, 15, 17‰, and so on. But since it is impossible to get water of an exactly determined salinity by means of distillation, one usually first obtains a concentrate, then, after filtering, the desired salinity is achieved by the addition of water.

If water with a lesser salinity than natural is necessary, then the concentrate or, better, natural water is

mixed with fresh water, and then the first formula is utilized (simple proportion). According to this formula one finds the quantity (milliliters) of natural water or concentrate x which is necessary for the production of one liter of water with the given salinity. Then, subtracting the resultant magnitude from 1000 ml, one determines the quantity of milliliters of fresh water needed for the mixing:

$$x = \frac{1000 \cdot c}{a},$$

where x is the number of milliliters of natural sea water or concentrate, a is the salinity of sea water or concentrate (in ‰), and c is the salinity of the resultant water (in ‰).

For example, in order to get 1 liter of water with a salinity of 12.5‰ from water with a salinity of 17‰ and from fresh water, one must take 264.7 ml of fresh water and 735.3 ml of concentrate. The course of reasoning is as follows. If one prepared 1000 ml of water with a salinity of 17‰, then one would take 100‰ of original sea water or concentrate with a salinity of 17‰, but in as much as one wants water with a salinity of 12.5‰, the following proportion is created:

17--100
12.5--x

then $x = \frac{100 \cdot 12.5}{17} = 73.53$ or 735.3 ml, if the calculation is done for 1000 ml.

For the preparation of water with a higher concentration than natural sea water, it is usual, in order to least disturb its salt content, to unite natural sea water with a concentrate derived from it. In this case a problem with

two unknowns is posed, and one uses the second formula:

$$y = \frac{1000(a-c)}{(a\ \ b)}$$

where y is the amount of natural water (in ml) which one must take for the preparation of water of a given concentration, a is the salinity of the original concentrate in %, b is the salinity of the natural water in %, and c is the salinity of the desired water in %.

For example, it is necessary to get 1 liter of water with a salinity of 17% from a concentrate of 40% and natural sea water of a salinity of 11%. According to formula 2 we find the amount of sea water necessary for the preparation of sea water of the desired salinity 17%:

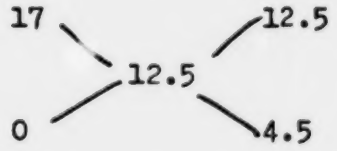
$$y = \frac{1000\ (40-17)}{(40-11)} = 793.1 \text{ ml}$$

In order to achieve a solution of necessary concentrate from two others one may use the "law of the cross." Briefly, it is the following: the cipher of the needed concentrate is written in the center of two intersecting lines, at the left ends of which are written the concentrations of the extant solutions, the larger above, the smaller below. Then, after subtracting the value of the given concentration from that at hand, the resultant difference is written at the free ends of those very same lines. The numbers gotten, written first above and below, show how many weight (or volumetric) units of each water it is necessary

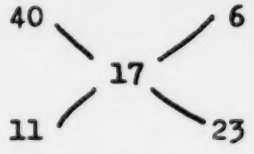
to take in order to achieve a solution of the desired concentration.

Examples of calculation.

1. The preparation of water with a lesser saline concentration than natural. In order to prepare water with a salinity of 12.5% from sea water of a salinity of 17% mixed with fresh water, one must mix 12.5 parts of sea water and 4.5 parts of fresh water.



2. The preparation of water with a greater saline content than natural. In order to prepare a water with a salinity of 17% from a concentrate with a 40% salinity and sea water of 11% salinity one must mex 6 parts of concentrate and 23 parts of sea water.



After the mixture of two different waters for the preparation of a given water, it is necessary to mix them and to confirm the salinity of the new mixture by titration, since during the mixture of large quantities of water it is possible to make errors and mistakes. In general, however, with careful calculation of the salinity of the original waters and the exact measurement of their volumes by graduates, graduated cylinders and measuring pipettes, one may calculate the salinity of the experimental waters

with an accuracy of 0.2-0.3% without especial labor.

The indicated accuracy is completely sufficient for the execution of biological (survival) and certain physiological (breathing) experiments. During the study of the osmosis regulating process in aquatic animals, however, it is necessary to check the content of salts in the water not only by titration, but by means of cryoscopy as well.

For convenience in the execution of serial experiments it is best of all to have previously prepared all of the waters intended for the experiments in satisfactory quantities. For example, for the parallel determination of the survival of invertebrate sea and fresh water animals in Azov water (beginning with fresh and ending with a concentration of sea water of 40-50%), it is necessary to have already prepared a series of Azov waters with the intervals 2, 2.5, and 5.0%. It is reasonable to prepare not less than five liters of each solution. During work with larger subjects, the volume of water is increased.

The preparation of water of each salinity is done in separate, but always marked containers, the row of which is distributed on a shelf above the experimental bench. In rounded off numbers on each vessel should be a clear inscription: the name of the water, its salinity, and the data of preparation. In each vessel is placed a special glass siphon.

A well washed rubber pipe is also harmless, but is destroyed in salt water.

The Determination of the Salinity of Water.

As indicated earlier, the salinity of water, prepared by any means, should be checked by titration with the help of an exact solution of $AgNO_3$.

$AgNO_3$ connects the ion Cl'; however, the determination of the general salinity is done by means of the multiplication of the indicators of chlorine, by the corresponding coefficient (see below), which shows the relation between chlorination and salinity of the given water (chlorine number).

For the determination of the salinity of waters it is best of all to use the micro-method put forth by prof. S. V. Bruevič (1944).[3] For the use of this method only 1 ml of experimental water is needed in all, and, consequently, one may calculate the concentration of salt in the smallest aquaria. The loss of silver in titration is minimal, and the titration apparatus is made portable. It is useful for work in the laboratory, as well as in field conditions.

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[3] S. V. Bruevič and S. K. Demenčenok, Instructions for the Preparation of Chemical Investigations on Sea Water, Moscow, Glavsevmorputi, 1944 Instrukcija po proizvodstvu ximiceskix issledovanij morskoj vody .

The recommended method allows one to determine the chlorine level of water with an exactitude of from 0.1-0.05%, which is completely satisfactory for biological goals.[4]

The apparatus for the titration and for the containment of the solution of $AgNO_3$ is very simple: a bottle of dark glass with a volume of about 2 liters, on the neck of which is placed a collar-holder for the burette. For the titration of salt waters one may use an exactly graduated pipette with a volume of 2 to 4 ml, attached under the burette, or micro-burette.

To be suitable for the micro-method the solution of $AgNO_3$ should contain 24.75 mg of pure $AgNO_3$ per liter, one ml of which corresponds approximately to 5 mg of chlorine. The titrate of silver is established in relation to normal water which contains 19.38‰ Cl'. Normal water is prepared by the laboratory of the Arctic Institute of Glavsevmorputi.

If for any reason normal water is not to hand, then the silver titrate may be determined by an exactly prepared solution, NaCl, which should contain 19.38‰ Cl'. For the preparation of one liter of such a solution one must take 33.15 g of dry NaCl.

The determination of the titre of the $AgNO_3$ solution

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[3] This method is not useful for the determination of the level of chlorine in fresh water and in water with a salinity of less than 1‰.

and saline level of sea waters is done in the same way and with a single pipette. A thin 1 ml pipette is rinsed several times in normal or experimental water, after which it is filled to the mark with the same water and emptied into a porcelain crucible (volume 15-20 ml) in an amount equal exactly to 1 ml. Then a single drop of 10% solution of K_2CrO_4 is added. The resultant mixture is titrated with $AgNO_3$, while being constantly stirred with a thin glass rod, until the transition of the green-yellow color to a very pale orange-yellow which does not vanish for 15 seconds during stirring. During titration of normal water, which contains 19.38% Cl', the loss of silver should lie within the limits of 3.847-3.906 ml. The solution of the latter is considered adequately exact if, during titration of one ml of normal water, the deviation of the concentration of silver from the standard does not exceed 0.05-0.06 ml or 0.15 ml with the use of a Knudsen burette, with the division of its volume into twin milliliters (see Bruevič and Demenčenok, 1944).

The calculation of the chlorine level and salinity of experimental water is done in the following way: the amount of $AgNO_3$ used in the titration tests is multiplied by five in consequence of the fact that 1 ml of solution corresponds approximately to 5 mg of chlorine. The value

received corresponds to the content of chlorine in the liquid under investigation. Having multiplied the resultant value by the corresponding chlorine coefficient, we determine the salinity of the water under investigation.

For example: into the titration of 1 ml of Caspian water of unknown salinity went 1.06 ml of $AgNO_3$. Having multiplied this number by 5, we get 5.3% Cl'; multiplying this by the chlorine coefficient--2.38--we get the value of the salinity: 12.6%.

The chlorine coefficients for the waters of our southern seas differ one from another because of various relations, and of the ions of the salts in the water of these basins (Table 1). Besides this, the chlorine numbers for the waters of each sea change somewhat depending on a non-constant salt content and the concentration of salts in the water of freshened regions, and on other reasons. For a more exact calculation of salinity in the waters of a given basin one introduces certain other constant values, at which point the chlorine number acquires an complex make-up (Table 2).

Having multiplied the chlorine coefficient by the indicator of chlorine of the experimental water, and having added the constant value (often this latter does not change markedly the salinity of the water, and is often omitted in biological experiments), we get the value of salinity in the waters of a given sea. The salinity of different seas with one and the same level of chlorine is different (Table 3).

Table 2

Chlorine Coefficients of Sea Waters

1 Водоемы	2 Коэффициент	3 Автор
a Океан	0,03+1,805 Cl'	Кнудсен и Серенсен (1902) a
b Черное море	0,18+1,795 Cl'	Кондырев и Юрьев (1950) b
c Азовское море	0,21+1,794 Cl'	Мусина и Микей (1955) c
d Каспийское море	— 2,386 Cl *	Океанографические таблицы для Каспийского моря под ред. Зенина В. А. и Шишмана В. А. (1949) d
. .	0,14×2,36 Cl' **	
e Аральское море	— 2,84 Cl'***	Степанов (1907) e
. .	0,26+2,791 Cl'	Блинов (1956) f

Key: 1-Water basin
- a) Ocean
- b) Black Sea
- c) Sea of Azov
- d) Caspian Sea
- e) Aral Sea

2-Coefficient

3-Author
- a) Knudsen and Serensen (1902)
- b) Kondyrev and Jur'ev (1950)
- c) Musina and Mikej (1955)
- d) Oceanographic Tables for the Caspian Sea, V. A. Zenin and V. A. Sisman (1949)
- e) Stepanov (1907)
- f) Blinov (1956)

Table 3

Comparative Magnitude of Chlorine Level (Cl‰), Salinity (S‰), and the Conditional Density for the Waters of the Aral and Caspian Seas, and the Ocean (According to Blinov)

Таблица 3

Сравнительные величины хлорности (Cl‰), солености (S‰) и условной плотности для вод Аральского и Каспийского морей и океана, $\sigma_{17,5}$ (по Блинову)

Cl ‰	1 Аральское море		2 Каспийское море		3 Океан	3 Океан
	S ‰	$\sigma_{17,5}$	S ‰	$\sigma_{17,5}$	S ‰	$\rho_{17,5}$
1,0	3,05	2,73	2,50	2,14	1,84	1,44
1,5	4,45	3,90	3,63	3,12	2,74	2,13
2,0	5,84	5,06	4,86	4,10	3,64	2,83
2,5	7,24	6,22	6,04	5,08	4,54	3,52
3,0	8,63	7,39	7,22	6,05	5,45	4,21
3,5	10,03	8,56	8,40	7,03	6,35	4,89
4,0	11,42	9,72	9,58	7,99	7,25	5,58
4,5	12,82	10,88	10,76	8,96	6,15	6,27
5,0	14,21	12,05	11,94	9,93	9,06	6,96
5,5	15,61	13,22	13,12	10,89	9,96	7,65
6,0	17,00	14,38	14,32	11,85	10,96	8,34
6,5	18,40	15,54	15,51	12,80	11,76	9,02
7,0	19,80	16,71	16,70	13,76	12,67	9,71
7,5	21,19	17,87	4 Измерения не производились		13,57	10,40
8,0	22,58	19,04			14,47	11,09

Key: 1- Aral Sea 2- Caspian Sea 3- Ocean 4- No measurements carried out

In connection with the fact that in long experiments the waters for experimentation are prepared many times and one series may somewhat differ re salinity from another, it is necessary to make an exact notation of the original indicators of each salt point in the series. For the registration of all this data a separate journal is indicated.

Table 4

Journal and Example of Entries for the Comparison of Waters of Varying Salinity

1 Дата смешения вод	2 Исходная вода: a название	S ‰	3 S ‰ заданной воды	4 Количество воды, взятой для смешения (мл)	5 n (количество $AgNO_3$, мл)	n·5 = Cl‰	K·Cl = S‰	6 Примечание
5. V 1955 г.	b Каспийская, концентрат . .	22,8	17	309,6	1,44	7,2	17,1	7 Хлорный коэффициент K = 2,38
	c Каспийская, естественная . . .	14,4	—	690,4	—	—	—	

Key: 1-Data of the mixed waters 2-Original water: a)name, b) Caspian water, concentrate, c) Caspian water, natural 3-S% of the given water 4-Quantity of water taken for mixing (ml) 5-Quantity of $AgNO_3$, ml 6-Notes 7-Chlorine coefficient.

It is desirable to have a table of the tests of the titre of the working solution of $AgNO_3$ in the very same journal.

Table 5

Journal of Notes on the Testing of the Titre of the Working Solution of $AgNO_3$

1 Дата	2 Количество нормальной воды (в мл)	3 n (количество $AgNO_3$ в мл)	n·5= Cl‰	K·Cl= S‰	4 Примечание
5.V 1957 г.	1	3,87	19,35	34,95	5 Хлорный коэффициент — K=0,03+1,805

Key: 1- Data
2- Amount of normal water (in ml)
3- Amount of $AgNO_3$ in ml
4- Notes
5- Chlorine coefficient

The Weighing of Living Experimental Animals

In the determining of the reaction of individual animals to the [illegible] of the [illegible], it is best of all to conduct experiments on mass material. For that reason, the weighing of animals, especially invertebrates, is often carried out in a group fashion. However, not all subjects endure weighing equally well. Fish (except for the larvae), molluscs, and many species of worms easily endure weighing in a dried-out form. These animals are taken from the aquarium with a net, dried on filter paper or clean linen,

and are weighed in a dry hour glass or in a box of known weight.

During the weighing of shrimp and other crustacea one must be careful to preserve the thin and fragile parts of their bodies (antennae, legs, and so on). If the animals are very tender and leave parts of their bodies on the filter paper (antennae, legs, and so on), then one must use decanting, during which the animals remain on one and the same piece of filter paper, and drier pieces are placed underneath for the drawing out of the moisture; after that, the animals are carefully transfered with a needle, lancet, or brush to the glass for weighing.

The procedure of weighing separate groups of molluscs must be done at the same time, since bivalve molluscs, on being disturbed, open their shells and intake into the cavity of the body a certain amount of water. During a long wait on the filter paper they from time to time open their folds and let out water from the mantle; from this it follows that their weight changes to differing degrees.

Fish older than very young are best weighed in a dry napkin on spring scales. After each weighing of the experimental object, the napkin's weight is checked.

Crustacea plankton and many other invertebrates, as well as the larvae of fish have a hard time with drying on filter paper, and the amount of water needed to keep and contain them often weighs more than they do, and to weigh them without drying and injuring them is impossible. For this reason there are a number of methods for the determination of the weight of a subject in solutions of sugar or other substances at a known degree of concentration (Gaevskaja, 1938; Levanidov, 1945). Usually these methods of weighing are clumbersome, and not always applicable. Under semi-field conditions animals weighed in solutions either lose their capacity for life and quickly die, or they become useless for experimentation.

In order to avoid injury to the experimental animals, one proceeds in the following way: given mass material, select an average sample (10, 20, 30 examples), weigh that, and then determine the average weight of a single sample. The animals weighed are not used in the experiment, but for that another exactly counted group of animals is prepared, the weight of which is determined by the weight of a single example.

And, finally, if it is necessary to know the exact weight of experimental animals (for example, during the determination

of the norm for the need for oxygen, food demands, and so on), and it is difficult to determine the number of experimental subjects (a massive number of zooplankton), and, consequently, it is impossible to utilize the average weight, then the animals are weighed after the experiment. In the course of this, as a rule, the material becomes unsuited for further experimental use.

In the study of the effect of the salinity of the environment on aquatic animals it must be remembered that their weight changes with the concentration of salts. For example, during the submersion of worms and molluscs into a hypotonic solution their tissues are irrigated and their weight increases; contrarywise, during the submersion of these very same animals in a hypertonic solution, they lose water and their weight decreases. The weight change may reach considerable magnitudes (up to 20% and more), and, therefore, during the weighing of experimental animals it is obligatory to preserve identity: to determine the weight of the animals which have lived in their natural environment, or only those which have lived under the conditions of the control experiment, while the weight of the remaining groups is determined by the mean weight of the control individuals. By measuring the weight of experimental animals placed in water of differing salinity one may follow the general course of the process of their

osmosis regulation. If the growth of individuals in connection with the conditions of salinity is determined, one must first get their weight after the equalization of their osmotic pressure in experimental conditions.

Vessels and the Preservation of Aquatic Animals in the Laboratory

In working with sea waters of varying salinity and other saline solutions one uses vessels made of materials that do not enter into reactions with the solution: 1) glassware: aquaria, aquarium jars, flasks, Petri flasks, bottles, and so on; 2) enamelware: tanks, buckets, dinner pans (which are very convenient for the transportation and holding of animals on the road, and which come available in 5 and 6 packs); 3) plexiglass: aquaria, tanks, special apparatus, and various types of plankton aquaria; 4) cemented aquaria and basins; 5) delft or glazed clay tubs, reservoirs, and so on; 6) polyetheline sacks. One must strickly avoid the contact of sea water with the metal parts of an aquarium, galvanized iron (buckets, basins), copper, lead, and so on. Aluminium vessels become corroded in sea water and may be used only for the temporary preservation of certain species, and that only with water with a very low salt content. The applicability of aluminium vessels for the holding of

fish and invertebrates should be confirmed beforehand by experiment.

For experiments with fish the most convenient aquaria are free flow aquaria of sheet glass, or aquaria made of a frame work with a cement bottom and metal slots. The aquaria may be of divers volumes and forms: oval, circular, square, rectangular and so on, from half a ton to two ro three liters. For the holding of pelagic and bottom fish (pike, bream, grey mullets, etc.) oval, circular and rectangular aquaria are desirable. For bottom fish (bullheads, flounders, etc.) the magnitude of the water level has less importance, and the aquaria may not reach a level of 25-35 cm in height (keeping in mind the dimensions of the experimental fish vary within a range of 10-25 cm).

The speed of water current in aquaria is established depending on the demands for oxygen of the fish, the temperature of the surroundings, and on the density of the experimental subjects. Usually pelagic fish (and invertebrates) are more active than bottom fish, and their demand for oxygen is larger and their oxygen level is also higher than in the seldom moving bottom forms.

Pelagic invertebrates survive relatively well in high, but not too narrow vessels (20-30 cm high with a diameter of 10-15cm), filled two-thirds full of water.

Bottom invertebrates (molluscs, worms, and other forms) survive best in flat and wide crystallizers with a low water level

not to cover the experimental subjects for more than 1 or 2 cm. Moreover, for certain species (worms and others) a ground is needed, for they build their tunnels in it, and there find food and refuge.

For the longterm holding of animals in aquaria, especially when one is trying to study their growth, development, etc., it is necessary to create the most favorable conditions:

to aerate well the water, to cultivate the necessary water plants in the aquaria, to feed daily with natural foods, and so on. However, the description of the methods of long term holding in salt and sea water is a specialized subject.

The Set-Up of Experiments.

The relation of aquatic animals to the action of a changing level of salt in the environment may be demonstrated by various methods. For example, 1) the determination of the survival of an individual of a species at one or another stage of a changing salt level in the water, 2) the study of the energy exchange and the osmosis regulatory process in the changing salt environment, and 3) the study of the distribution of individuals in nature in relationship to differing levels of salinity.

The indicators established by these means permit one to more or less accurately establish the salt tolerance

of a given population. However, from one of these indicators it is not always possible to determine the relation of the subject under investigation to salinity. And for this reason one often has to carry out experiments to study this factor on individuals, and on various ecological groups. One uses experimentation and observation in nature.

In experiments one uses a methodology founded on the effect of a sharp change in salinity on the organism and its physiological adaptation to waters of differing salinity, while in nature one uses long observations on the fluctuations in the area of a population which lives in various water basins, on in varying salt conditions in the environment.

One usually studies the salt resistance of an individual of separate species by a more or less sharp change in the salinity of the environment. But not all animals equally well withstand the shock of a sharp change in salinity. We have especially noticed this in forms with a passive osmosis regulation (molluscs) which are subjected to the action of sea waters with a large content of bi-valent ions: the Caspian and Aral (Karpevič, 1955). Sometimes the transplanting of animals from weakly salt water to a stronger concentration leads to their deaths not because they are incapable of "assimilating" that concentration of salt, but

because the saline increase is too large for them. In such cases it is obligatory to use a gradual method of habituating the animals to a change in the salinity of the environment.

We shall discuss in more detail the conducting of experiments with a sharp and a graduated change in the salinity of the inhabited environment.

The Survival of Fish and Invertebrates Under a Sharp Change in the Concentration of Salt in the Environment.

In a series of identical vessels one pours already prepared sea water (Azovian, Caspian, Aralian, or some other) with a salinity approximately 0 (fresh water), 2.5, 5.0, 7.5, 10.0, 12.5%, and so on, right up to the estimated lethal level. In these vessels are placed groups of counted, weighed animals which, before the experiment, have lived in their natural environment: for example, in Caspian water with a salinity of 5%. In this case the vessel with the Caspian water (S=5%) is the control, and all the rest are the experimental vessels.

If molluscs are being investigated, placed in crystallizers of a diameter of 16-17 mm and a depth of circa 6 mm, for 15-20 g of living weight it is sufficient to pour in 300-500 ml of water. For some molluscs with a weight of

circa 0.25 g (5-7 individuals) it is best of all to use cylindrical vessels with a height of 17-20 cm and a diameter of 15-17 cm, filled with 1-1.5 l of water.

The series of experimental vessels should be identical as to form and volume, and should be kept in the aquarium area under more or less identical conditions. The most favorable conditions for almost all animals of the temperate zone are the following: constant temperature (16-18°), scattered light, the absence of sharp knocks, the raising of humidity in the area (to lessen the evaporation of salt water) , the periodic aeration of the aquaria, and so on.

During the holding of animals in such conditions it is sufficient to check the aquaria once every 24 hours in order to follow the condition of the animals, to feed them, count them, remove the dead, measure the temperature, remove the water when necessary, or to only aerate the aquarium, and also to check the changes in salinity. In aquaria covered with glass, or in aquaria in a place with a relatively high humidity, evaporation happens slowly, and the salinity changes insignificantly in the course of the experiment. In dry rooms and in small, flat crystallizers

the water evaporates easily and, consequently, the salt content may change even in the course of twenty-four hours considerably.

With a higher or varying temperature, or in the case of a high mortality of animals, one must check the aquaria not less than twice in 24 hours, and even change the water. Especially important is the frequent checking of the animals in the first few days after the beginning of the experiment, since in this period is observed the largest loss due to trauma.

The observations are made very exactly and quickly: for each series of experiments a card or table is made, where everyday at determined hours are entered notes on the number of animals living or dead, while all other observations are entered in a diary with an indication of the date, the number of the series, and the number of the crystallizer (Table 6).

Example: Experiment No. 1. Survival of Dreissena andrusovi in Caspian water of differing salinity (sharp change in salinity).

The experiment was begun on May 17, 1955. The experimental animals were taken with a drag in the Zaburun'ja region (northern Caspian) the 10th of May, 1955, in water with a salinity of 5‰ at a temperature of 18°, at a depth of 2 m; the floor was sand with gravel. Before the experiment the Dreissena were kept in natural water, spawning was not observed, although the individuals were adult (length 7-16mm), in clumps. 10 clumps were taken (Table 6).

Table 6

Example of the Results of an Experiment

1 S (в °/₀₀) в опыте	2 Количество подопытных животных (в экз.)	3 Вес животных (в г)	4 Количество погибших животных				6 Длительность пребывания в воде данной солености (суток)	7 Погибло за время опыта		8 Среднесуточный процент гибели
			5 Температура и время наблюдений					экз.	%	
			18° (11.V)	17° (12.V)	16° (13.V)	и т.д.				
0	100	—	10	8	10	—	10	100	100	10,0
2,5	100	—	5	2	0	—	20	10	10	0,50
5,0	100	—	5	0	0	—	20	7	7	0,35
7,5	100	—	3	2	0	—	20	5	5	0,25
10,0	100	—	5	6	0	—	20	11	11	0,55
12,5	100	—	10	6	5	—	15	30	30	2,0

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Key: 1-S (in ‰) in the experiment
2-Number of experimental animals
3-Weight of the animals (in g)
4-Number of dead animals
5-Temperature and time of observation
6-Duration of presence in water of the given salinity (in 24 hour periods)
7-Died during the experiment
8-Mean procent of deaths per 24 hours

Experiments to determine the survival of animals in water of a given water basin, but with varying salinity, should be made as long as possible: for fish not less than 2-3 months, for crustaceans 3-4 weeks at a temperature of 18-20°, for molluscs not less than one or one and a half months, and so on. For each species one may find the physiological criteria for the duration of the experiment. Adult individuals of many species have a physiological resistance and may be located in sub-lethal conditions for a long time, but may not survive this environment constantly. Therefore, in order to achieve reliable results re their reaction to the concentration of salts, one must make pains taking observations on the changes in their physiological condition: for example, pike may withstand a salinity higher than 18‰ for several weeks, but during this their eyes grow dim, they become blind, and die. Mytilaster lineatus survive for 10-12 days in a hydrogen-sulphide environment (Karpevič, 1940). The closely closed shell protects them from unfavorable external circumstances. Gas exchange in these conditions is at a depressed level, and a considerable amount of the products of exchange remain un-oxygenated, and an excessive build-up of the latter leads to death.

In view of the fact that the critical periods are not known for all animals, one must discover them in the course of experiment, lengthening the time of observation as much as possible.

The results of experiments are most clearly etched after a full count of the number of live and dead individuals after the full course of the experiment (in % of the original number of individuals). With the necessity of comparing the results of various series one must subordinate the achieved results to a single time frame. We suggest that the dead be counted every 24 hours, and expressed in percent (the "mean twenty-four hour percent of death"). This indicator is convenient to use and is usually applied by us for the construction of graphs for the survival of experimental subjects.

In order to achieve reliable results it is necessary to carry out two or three series of experiments in parallel, but sometimes, with careful preparation, a high level of survival, and good conditions in the aquarium room, it is enough to carry out only one or two series.

The Survival of Fish and Invertebrates Under a Gradual Change in the Concentration of Salts in the Environment ("Gradual Change of Salinity")

The group of experimental animals (all, except the control group) are transferred from their natural water

into vessels with water which is minimal in relation to the change in salt concentration from the natural water. The difference in the concentration of salts between the natural water and the experimental water is chosen in dependence on the tasks of the experiment and the resistance of the experimental subjects. For example, fresh water molluscs (Anodonta piscinalis) close their folds during a change from fresh water to water with a salinity of 3-5‰, but during a rise in salinity of only 1‰ the folds remain open. Consequently, a salt jump of 1-2‰ (or 0.6‰ Cl' for azov water and 0.4‰ Cl' for Caspian) is completely possible for them. Salt water molluscs easily survive a change in concentration of salts of 2-2.5‰. Molluscs from the sea, crabs, and some worms survive a salt jump of 5, 10, and even 15-20‰. For a more exact determination of the critical salt concentration for a species we recommend the maximalization of the salt jumps in a series, with a variance between them of not more than 5‰. In conditions sub-lethal for a given species more fractionalized transitions from one salinity to another are necessary (a transition of 0.2-1‰).

The experimental animals must be left in each solution until the osmosis regulation equalization of the internal fluids and the external environment is finished. In each species this process proceeds at its own tempo, the determination of which is often very difficult. In such a case

one must increase the time period of the maintaining of the animals in the given environment. For example, a 48 hour holding of molluscs in a new environment is more than enough for the osmosis equalization of their internal and extermal surroundings, and for crustaceans 12-20 hours is fully suffieient.

After the holding of the experimental animals in the first environment, they are put in solutions of the successive concentrations, leaving one group in each of the previous "salt point" solutions (Table 7).

Thus a series of vessels with all salt concentrations is formed, from 0 to the thresh-hold salinity, with identical intervals between them.

Example: Experiment No 2. Let us suppose that May 18 were taken 8 groups of Dreissena, which formerly lived in Caspian water with a salinity of 5‰ (see Table 7); the 20th of May all groups, except No 3, were placed in water with the nearest salinity, i.e., 2.5 and 7.5‰, and after 48 hours they were placed in the following two "salt points," and so on. When all groups were distributed in the corresponding vessels, the observations on their survival and the registration of the living and the dead were done just as in experiment 1 (Table 6), but during the calculations of the survival or percent of death the entire period of their presence in water of a given salinity is taken into account.

The Methodology of Determining the Oxygen Demands of Water Organisms in Waters of Varying Salinity. (Respiration Experiments).

Table 7

Experiment No. 2. The Process of the Physiological Adaptation of Dreissena Which Lived in Caspian Water with a Salinity of 5‰ to Caspian (Blacksea or other) Waters of Varying Salinity (The Notes on the Experimental Material Are Noted as in Experiment No. 1)

1 Солёность воды в опыте (‰)	2 № групп животных и их выживание при адаптации к водам разной солености												4 Результаты адаптации		
	19. V (20°)		20. V (21°)		22. V (20°)		24. V (20°)		26. V (22°)		28. V (22°)		5 Пребывание в данной солености (сутки)	6 Гибель	
	№	3 Количество животных	№	3 Количество животных	№	3 Количество животных	№	3 Количество животных	№	3 Количество животных	№	3 Количество животных		количество (экз.)	%
0	—	—	—	—	1	10	1	8	1	8	1	6	6	4	40
2,5	—	—	1—2	по 10	2	10	2	9	2	9	2	8	8	2	20
5,0	1—8	по 10	3	10	3	10	3	10	3	10	3	9	10	1	10
7,5	—	—	4—8	по 10	4	10	4	10	4	10	4	10	8	0	0
10,0	—	—	—	—	5—8	по 10	5	10	5	10	5	10	6	0	0
12,5	—	—	—	—	—	—	6—8	по 10	6	10	6	9	4	1	10
15,0	—	—	—	—	—	—	—	—	7—8	по 10	7	9	2	1	10
17,5	—	—	—	—	—	—	—	—	—	—	8	8	0	2	20
и т. д.	—														

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Key: 1-Salinity of the water in the experiment (‰)
2-Number of the groups of animals and their survival during adaptation to waters of varying salinity.
3-Number of animals
4-Results of adaptation
5-Presence in a given salinity (in 24 hour periods)
6-Deaths
7-Number of subjects

In field conditions or in experimental-field laboratories one usually determines one side of the breathing of aquatic animals, viz. the intensity of their oxygen demand. The determination of animals re CO_2 is not persued, due to the relative complexity of the determination of the latter.

At the present time there are many original methods for the determination of the intensity of oxygen demand by water animals (Krog, Gross, Barkroft and others). We shall dwell only on the simplest and most reliable under field conditions of these methods: that of closed vessels, called, not entirely accurately, the Winkler method. The other, more delicate methods used for physiological investigations, are not always capable of giving reliable results in the hands of the biologist. A critical assesment of them is given in the list of G. G. Vinberg (1956).

Vessels and Hardware.

For experimentation are used calibrated, wide-mouthed jars with a flat and wide bottom, and with a ground-in stopper ("respirators") without fluting. The volume of the jars may be diverse, fomm 100 ml to 5-6 l depending on the dimensions and number of the subject animals. For the study of the breathing of small plankton animals it is best of all to use respirators with a volume of 100-200 ml; for small molluscs, worms, larvae jars with a volume of 500-600 ml are more convenient; for young fish, large molluscs

and crabs jars of a volume of 1.5-2 l and so forth. For relatively large and easily excitable fish it is best to use glass aquaria (round, rectangular, or cubic forms) with glass attached (instead of roof coverings).

Especially convenient for the investigation of bullheads, sea ruff, and other fish are rectangular glass aquaria of a volume of 5-10 l placed exactly horizontal on the aquarium table.

The content of oxygen in the water is determined by the Winkler method. The application of this method during experimentation with sea waters has been described in detail by Prof. S. V. Bruevič and S. K. Demenčenko (1944). They have shown the methods for the preparation of the necessary reagents and have described the vessels for the preservation of solutions, pipettes, and so on.

The Preparation of the Animals for the Experiments.

Depending of the goals, the individuals of various species are prepared in different ways.

Hardy invertebrates and fish, which withstand laboratory conditions well, and also individuals at a resistant stage of development, are sorted into groups several hours or days before the experiment and are held in separate aquaria under usual or natural conditions of the physico-chemical environment.

Usually individuals are chosen according to the developmental stage, their size, or according to sex, and so on. During ecological experiments arises the necessity to determine the general demands for oxygen of any population which consists of individuals of varying size. In such a case in a sample selection one determines the percent relation of separate size groups, and the mean weight of a single individual of each of these groups.

Animals which are especially sensitive to changes in the environment are selected and counted immediately before the experiment and are at once distributed among the jars. Here may be placed developing sturgeon, zooplankton organisms, some crustaceans, worms, and so forth. To traumatize adult fish immediately before an experiment is also not desirable, since in the first hours after transplantation or weighing their exchange changes sharply. Consequently, fish must be prepared not less than 2-4 hours before the beginning of an experiment. It is even better to establish the intensity of their gas exchange without any kind of transferal, under conditions usual for them, using aquaria in the capacity of respirators.

It is desirable to weigh invertebrates immediately before the experiment, or immediately after its termination.

If it is necessary to achieve data on the intensity of exchange in hungry animals, one must stop feeding before the

experiment and keep them without food until evacuation of the intestines. The period necessary for this varies depending on the preliminary feeding of the animals, the duration of digestion, and the temperature of the environment. In many invertebrates the intestines are freed in the course of several hours (2-12) at a temperature of 18-20°, and, consequently, the holding of these for a space of days in filtered water garantees the purity of the experiments.

The duration of digestion in different species of fish varies strongly: in carp and some bullheads, and others, the time of the process of food through the intestines at 18-20° exceeds one or two 24 hour periods, while its effective digestion occurs even more quickly (the effective digestion of food ends after the dissolution of the most caloric and nutritious muscular and fat portions of the food; see A. F. Karpevič and E. N. Bokova,"Tempy perevarivanija u morskix ryb," Zoologičeskij žurnal, t. XV, vyp. 1, 1936 (č. 1), t. XVI, vyp. 1, 1937 (č. 2) [Tempo of Food Digestion in Sea Fish," Zoological Journal, v. XV, fasc. 1 (part 1), v. XVI, fasc. 1 (part 2)]). In pike, ruff, sturgeon, sardines, and many others, especially in predators, the period of effective digestion may last several days (more than 2). Such fish must be kept without food for 48-96 hours, or more, depending on the conditions

of temperature and their prior feeding.

The indicator of gas exchange in hungry and immobile fish may characterize their basic exchange. However, during ecological studies it is important to know the intensity of gas exchange in normal, active, feeding, and growing organisms (normal life metabolism), and, therefore, it does not follow to let the subject animals hunger for long before respiration studies. One should not, also, feed them just before an experiment, since in fish that have just been fed the intensity of oxygen demand sharply rises (Karpevič, 1958).

The Preparation of Waters for Respiration Investigations

The river or sea water intended for respiration studies is prepared 10-12 hours before the beginning of an experiment. All procedures during the setting up of an experiment occur in the following order: 1) the salt solutions of the necessary concentration are prepared; 2) these are filtered through a cotton or paper filter in order to separate organic or inorganic contaminants; 3) the solutions are aerated during filtration, letting the water in a thin stream through a high column of air, or injecting a stream of air into the aquarium. Then the water is poured into bottles high glases (water reservoirs) and left not less than 10-12 hours in the container to be used in the experiment. During this

possible) are placed in the respiration vessels along with a small amount of water. Then the vessels are filled up to the top with a siphon with the water intended for the experiment. It is very important that one end of the siphon be located in the middle or in the lower part of the water reservoir (one must not put it on the bottom, since it may catch up particles), while the other end should be on the bottom of the respirator. It is necessary to make sure that, during the filling of the respirator, the water not be mixed up and that there is no additional enrichment of oxygen from the air. The water, rising upward, should only come into contact with the air with its upper surface. These conditions may be satisfied with the use of a relatively narrow siphon (less than 5 mm in cross-section); then the stream of water will flow without too much turbulence.

During the filling of the respiration vessel one must also take care that the water, in which the animals are carried and which rises as the vessel is filled, flow out into a cuvette, and that there be no water bubbles or any kind of contamination.

After filling, the vessel is closed with a ground in stopper wetted with water, or a glass (if it is an aquarium), and placed in a bath with the same temperature as the water. With the following of these precautions, one almost never gets bubbles in the respirator, especially if the

experiment is carried out under a relatively constant temperature. One cannot allow a temperature change larger than 1-2° during the course of the experiment.

At the same time as the filling of the respirator jar, or immediately thereafter, one uses the very same siphon to fill both the control vessel without animals and two oxygen hour-glasses. The oxygen in them is fixed by Winkler's method (see above). According to the oxygen concentration in the hour-glasses one determines the concentration in the respirator at the beginning of the experiment. (The initial content of oxygen in the respirator may be fixed at the end of the experiment by the content in the control.) If the experimental subjects are animals that do not move much (molluscs, worms, bottom fish, etc.), the respirator is carefully turned during the course of the experiment and several times towards the end, in order to equalize the distribution of oxygen throughout the entire water volume:

The fact is that animals which move little first of all absorb the oxygen in the nearest layers of water, and with out the water's circulation around them there occurs an oxygen defficiency even if there is a high content of oxygen in the entire vessel. After the closing of such a respirator one may get incorrect results as to the presence

of oxygen and the demand of the subject animals towards the gas regime. Freely swimming animals (crabs, fish, etc.) themselves leave the depleted water and mix it up in the vessel.

The observations at the end of the experiment are carried out in the following order: 1) the time of the opening of the respirator is noted; 2) At opening, it is at once emptied by a siphon and put into prepared hour-glasses (their number and volumes are entered into the journal); 3) after the filling of the glasses a thermometer is put into the respirator, and the oxygen content of the hour-glasses is fixed; 4) after the determination of the water temperature, tests are made for the determination of the pH, oxidability, the animals are counted and weighed, etc. By the concentration of the oxygen in the test is determined the content in the respirator at the end of the experiment.

If an aquarium is used, one acts in the following manner: before the experiment in an aquarium of known volume, which already contains fish, one causes the water to flow and lets it go right up to the complete change of water in the aquarium. Then, by the usual methods, one determines the oxygen content in the aquarium, and only after this one decreases the flow of water, while the siphon is put in some corner or other. Leaving a gap for the siphon, one carefully puts on the glass cover while, at the same time, taking out the siphon. With some practice the aquarium may be filled without leaving a single air bubble behind.

The method described permits one to determine the norm of oxygen demand simultaneously for many individuals in a quiet state. At the same time the individual variations in the intensity of breathing are levelled out, as well as the influence of fear and unnoticed damage to the individual. The method of covered vessels is simple and is easily mastered by biologists. But it is not too good for precise physiological studies, and mistakes may be made in its use.

In order to avoid many of these mistakes, one must take the following into account:

1) The content of oxygen in the reservoirs should not be lower than 80% of normal, as shown in the oceanographic tables (Table 10) for the given conditions of temperature, partial pressure, salinity, and so on.

2) During the determination of the oxygen demand of animals, the content in the respiration jar should be not less than 50% of normal. One must consider that the "critical" concentration of oxygen for certain animals occurs at a relatively high level in a vessel. At the critical concentration of oxygen in the water the subject organisms begin to feel its lack, their breathing becomes labored, and a temporary excitation is possible, which leads to an increased demand for oxygen. The "norm" established under such conditions will be incorrect.

3) When selecting animals for experimentation one must not injure them; in the course of the experiment one must look after their condition. It is important that the movements of the subjects and of their breathing organs be rhythmical and calm (the oscillation of the gills in fish, the movement of the siphon in molluscs, etc.). One must remember that hungry and immobile individuals use less oxygen than full and active animals. The oxygen demand of healthy, active, and fed animals, of females with off-spring, males, the yound, etc., is higher and changes depending on their physiological condition.

4) The weighing and tranfer of animals taken from their usual places of habitation destroys their normal behaviour at the beginning of an experiment: they begin to die and cease their normal breathing actions, or they swim in excitation, and their breathing rhythm increases, which leads to a considerable change in the indications of their oxygen demand. In order to minimalize the effect of these disturbances it is necessary to lengthen the period of respiration study for several hours.

5) When lengthening the exposition one must equalize the volume of the vessel, the weight of the animals, and the temperature. For example, in a vessel of circa 530 ml one may place not more than 15 g of molluscs or 0.5-1 g of crustacea, and the experiment may not last longer than 5 hours (with a favorable salinity and temperature of 20-21°).

6) If the animals comprize more than 0.5% of the volume of the respirator vessel, then when calculating the oxygen content the volume of the animals must be subtracted from the volume of the vessel.

7) With an excessive weight of subject animals the demand for oxygen becomes quicker and more unequal: in the layers of water surrounding the animals the oxygen quickly becomes exhausted, CO_2 builds up with the products of waste, and the pH decreases. All this may inhibit the intensity of gas exchange in animals, and even cause their death (especially in immobile and inactive forms: molluscs, etc.). In order to avoid the unequal distribution of oxygen in the jar, one must turn it several times in the course of the experiment.

8) It is obligatory to carefully, after the instructions of Bruevič (1944), select, titrate, and fix tests for oxygen. Because of the small volume of the hour-glasses the slightest carlessness in the procedure increases the mistakes in an experiment.

Moreover, if the water is contaminated by the products of nitrogen exchange, iodine is liberated during the course of the oxidation processes in the water. At titration of such a sample the starch present in it becomes blue, and to determine the end of titration is impossible. In order to avoid an extreme build-up of nitrogen products it is desirable to carry out respiration experiments with a

reasonable weight of animals and with pure water. If it is necessary to use water already dirtied, then one must use the method of the titration of iodine in the presence of nitrates worked out by B. A. Skopincev and O. V. Mitjagina (1939), and it is also obligatory to determine the oxidability and pH of the water (Bruevič and Demenčenok, 1944).

8) During the course of lengthy respiration experiments it is obligatory to use control jars. They are filled with experimental water (without animals) and kept under the same conditions as the experimental jars.

By the change in the oxygen content of the control jars one judges the quality of the water, the processes that happen in it, and introduces the corresponding corrections into the calculations of the oxygen demand of animals.

Respiration experiments, as experiments on the survival rate, should be carried out with the "sharp" and "gradual" changes in salinity. In the first case one proceeds in the following manner.

Out of the natural saline environment of the animals, they are placed temporarily in waters of varying salinity: 0, 2.5, 5, 7.5, 10%, and so on. After the determination of the intensity of their breathing each group of animals is replaced under their usual living conditions. If even part of them die during the experiment, the indicator of

the intensity of their breathing may be incorreet. Such a series permits one to determine the magnitude of the salt jump which influences the gas exchange of fish or invertebrates, and also the optimal salt zone for their respiration, but the general salt tolerance of the animals may not be established.

In the second case, i.e., with physiological adaptation to waters of varying salinity, one acts in the following way. At first the animals are put in water of increased or decreased salinity, and then the intensity of their respiration is determined. In this case the general salt range in which respiration is possible is established.

A journal of notes on respiration experiments is shown in Table 8.

The Calculation of the Results of Respiration Experiments.

The quantity of dissolved oxygen in natural water is usually expressed in cubic centimeters (at the present time in milliliters--ml) contained in a liter of water at a given temperature, under 760 mm of pressure. The magnitude of oxygen demanded by aquatic animals is often expressed in cm^3, and in ml, and in mg, etc. We recommend the use of volumetric indicators (ml), since this easily allows the determination not only the absolute quantity of oxygen, but also the percent

Table 8

A Table of Notes on the Determination of Oxygen Demand by Aquatic Animals in Sea (Name of Sea) Waters of Differing Salinity

For the experiment are taken (name of animal) by means of (name of tool) in the region_____, at a temperature of _____, salinity_____, depth_____meters, on a floor of _____, in a mass, with plants, etc.

The length of the subject_____, sex_____, age____

1 Название животных	2 № опыта	3 Дата	4 Количество животных	5 Вес (в г)	S (в ‰)	6 Объем респиратора (в мл)	10 Начало опыта: 7 Время (в часах)	8 Температура	9 Пикнометр: №	9 а объем (в мл)	11 Количество гипосульфита (в мл)	12 Кислород (в мл): а в пикнометре	12 Кислород (в мл): б в респираторе	в 1 л	13 % насыщения O_2	14 pH воды

Продолжение табл. 8.

16 Время	17 Температура	15 Конец опыта: Пикнометр №	Пикнометр а объем (в мл)	11 Количество гипосульфита (в мл)	12 Кислород (в мл): а в пикнометре	12 Кислород (в мл): б в респираторе	в 1 л	13 % насыщения O_2	14 pH воды	18 Длительность опыта (часы)	19 Общее потребление O_2 (в мл)	20 Потребление O_2 (в мл) на г. час)	21 Примечание

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Key: 1-Name of animal 2-No. of experiment 3-Data 4-Number of animals 5-Weight (in g) 6-Volume of respirator 7-Time (in hours) 8-Temperature 9-Hour-glass; a) volume (in ml) 10-beginning of experiment 11-Quantity of hyposulphate (in ml) 12-Oxygen (in ml); a) in hour-glass, b) in respirator 13-% of oxygen saturation 14-pH of water 15-End of experiment 16-Time 17-Temperature 18-Duration of experiment (in hours) 19-General demand for O_2(in lm) 20-O_2 demand (in ml/g, per hour) 21-Notes

of its saturation according to the oceanographic tables of Zubov (1931), and the data of Table 10. The weight of 1 ml of oxygen is equal to 1.429 mg.

As shown in the instructions of Bruevič and Demenčenok (1944) a solution of hyposulphate ($Na_2S_2O_3$) is useful for the titration of oxygen samples, and is prepared 0.02_n. In this case 1 ml of solution corresponds to 1 ml of 0.02_n solution of iodine or $\frac{8}{50}$ mg of oxygen.

Since in the determination of the demanded oxygen one often uses a hour-glass of small volume (30-50 ml, and not 150-160 ml as envisioned by the instructions), for the lessening of mistakes one makes a solution of hyposulphate half as strong, i.e., 0.01_n. If the solutions for the determination of its titre--KJO_3 and $K_2Cr_2O_7$ are prepared at the same concentration (0.01_n), then the final result of the experiment--the demand of oxygen (or its content in the water at the beginning of the experiment)--have to be decreased two times.

Below we will in full carry out the calculation of the determination of the content of O_2 in 1 liter of water according to the instructions of Bruevič and Demenčenok (1944).

The quantity of oxygen A, dissolved in 1 liter of sea water, expressed in ml, is determined by the formula:

$$A = \frac{0.112 \cdot n \cdot K}{V_2 - 2} \qquad (3)$$

where n is the number of ml of 0.02_n solution of hyposulphate gone into the titrate sample; K-the corrective coefficient of the hyposulphate solution--the relation of a precisely prepared solution of 0.02_n KJO_3 or 0.02_n $K_2Cr_2O_7$ (the method of the determination of the titrate has been described by Bruevič and Demenčenok, 1944) to the loss of 0.02_n $Na_2S_2O_3$ gone into titration; V_2 is the volume of the oxygen hour-glass in ml; 2 is the volume of the reagents added for the fixation of oxygen (1 ml of $MnCl_2$ plus 1 ml KJ in an alkaline solution--if one uses an hour glass with a volume of 30-50 ml it is suffiecient to add 0.3-0.5 ml of each of these reagents); 0.112 is the content of oxygen in 1 ml of water at 0° and 760mm of pressure (8/50.1/1,429=0.112).

If it is necessary to determine the disolved oxygen in a respirator, then one must calculate its volume, and then formula (3) will take on the following form:

$$A = \frac{0.112 \cdot n \cdot K(V_1 - V_3)}{V_2 - 2} \qquad (4)$$

where V_1 is the volume of the respirator in ml; V_3 the volume of the animals in the respirator. The remaining symbols are as in formula (3).

Usually for the determination of the titre of $Na_2S_2O_3$ one takes 25 ml of 0.02_n solution of KJO_3; one may take less, but not less than 10-15 ml. In this case it is necessary to use and thin and narrow burette, the volume of the graduated portion of which should not exceed 10 ml.

Example. For 10 ml of KJO_3 are used 9.62 ml of $Na_2S_2O_3$: then the corrective coefficient of the latter (K) is equal to 1.038, i.e., the working solution of hyposulphate is stronger than the standard by 0.0038.

Example of calculation. 12 animals with a mean weight of 0.98 g are placed in a respiration vessel with a volume of 555 ml. The respirator is filled with Caspian water with a salinity of 2.1‰. The experiment is carried out at a temperature of 22°.

For the determination of the dissolved oxygen an hour glass with a volume of 31.4 ml is used. Into it is poured 0.5 ml of a solution of $MnCl_2$ and 0.5 ml of an alkaline solution of KJ, then the glass is closed and shaken several times. After percipitation and the thickening of the sediment formed, it is dissolved in concentrated HCl, adding 1-1.5 ml to each sample, after which it is titrated with hyposulphate.

Into the titration of the contents of the glass goes 1.67 ml of 0.02_n solution of hyposulphate, the correction of which is K=1.01. Calculation by formula (4) shows: in 555 ml of water in the respirator is contained 3.45 ml of oxygen.

$$A = \frac{0.112 \times 555 \times (1.67 \times 1.01)}{31.4 - 1} = 3.45 \text{ ml}$$

Or 6.21 ml of oxygen in a liter.

By the oceanographic tables of Zubov (1931, p. 149), or by Table 10 we find: at 22° and S=2.0% in 1 ml of water is dissolved 6.28 ml of oxygen, which corresponds to 100% saturation. Consequently, in our respirator the water is 98% saturated.

At the end of the experiment, after 8 hours, 1.81 ml of oxygen is discovered in the water of the respirator, or 3.31 ml/liter, which corresponds to 52% saturation. Consequently, for 8 hours of the experiment 0.98 g of animals demanded 1.64 ml of oxygen.

Given the necessity of comparing the results of separate experiments the necessity of unitizing the weight of the organisms and the times of the experiments arises. The oxygen demand of small animals (invertebrates, larvae, fish and so on) is conventionally taken as 1 g of their weight to one hour (grams/hour), while for large animals one uses kilograms/hour (in no case may one limit oneself to the indicator of oxygen demand for a simgle organism without considering its weight).

Calculation by formula (4) is possible in the case that the change in the content of oxygen occurs on account of the animals respiration, and that there are in the water no processes which would noticable affect the oxygen content. But often in a insufficiently cleaned and filtered water small forms of vegetation may be present (certain diatoms may go through even paper filters, and are kept out, like bacteria, only by membrane filters), as well as organic

remains and bacteria. In the presence of water plants the oxygen content may increase, and in the presence of organic decay the content may fall. In connection with this it is necessary to set up a control, and if the difference in the oxygen content for one hour reaches 0.05-0.06 ml, it is necessary in calculation to take this into account, since over several hours (5-10) the decrease or increase of oxygen in the water may tell on the final results of the experiment.

If the oxygen content increases in the respirator (we assume that there is vegetation in the water), this increase (during the time of the experiment) is added to the magnitude of the mean demand for oxygen by the animals living in a parallel respirator. One must take care that the duration of the exposition in both vessels be identical, and that all other conditions--temperature, light, salinity, and so on--likewise be the same.

If the oxygen content decreases in the water of the control vessel, this value is subtracted from the mean quantity of oxygen used by the subject animals.

In conclusion, we shall introduce the full formula (5) of the calculation of the demand for O_2 in a closed vessel:

$$X = \frac{(A-B) \pm C}{P \cdot t}$$

Where A = the oxygen content in the respiration vessel at the beginning of the experiment (in ml); B is the oxygen content in the respirator at the end of the experiment (in ml); P is the weight of the animals (in g); t is the duration of the experiment (in hours); C is the difference in the oxygen content at the beginning and end of the experiment in the control vessel (in ml).

In ecological studies one must calculate the amount of demanded oxygen by the units of the live weight of the subject, including all skeletal formations (shells, carapaces, etc.). These formations arise in consequence of the life activity of the individual, and without them the existence of the animal is impossible. Only for specialized goals, when one already knows the oxygen demand per unit of live weight, may one determine the magnitude of the O_2 demand per unit of dry weight, per unit of weight of the soft parts of the body excluding the skeletal formations, for a single individual, etc.

The Method of Determining the Critical Concentration of O_2 and the Oxygen Threshhold for Fish and Invertebrates

In order to get a fuller idea of the characteristics of a species, and most often with practical goals, one endevours the determine not only the demand of an individual for O_2

but also the least quantity of dissolved O_2 with which it is possible to satisfy energy demands. These clues are especially important in the calculation of the allowable density of fish and invertebrates in different basins, aquaria, and reservoirs, as well as in the prediction of fatal phenomena, and so on. In the case of the selection of a form for acclimatization one of the most basic ecologo-physiological indicators may be the intensity of gas exchange and the sensitivity of an individual to a deficit of oxygen. (Any decrease in the content of dissolved oxygen in comparison with the norm is a deficit. However, a decrease in the content of O_2 of more than 50% of the norm may be dangerous for the existence of an animal). In the new conditions for a transferred animal may exist zones of periodic worsening of the gas regime, and one must know beforehand to what extent this is dangerous for their survival. For example, in the deep zones of the northern Caspian, in the central parts of the Azov and Black seas, in the Kursk inlet of the Baltic sea and in other sea and fresh water basins with clay bottoms, often arises anerobic conditions, and the oxygen content in the bottom layers falls to null. In such cases "famine", i.e., the death of animals from a lack of oxygen is inevitable.

The strength of the famine is determined by the resistance (duration) and magnitude of the deficit, and by

the demands of the species for oxygen.

At the present time data is gradually being gathered on the oxygen content, at which begins: 1) chocking off of the respiration in fish and invertebrates--the "critical content," and 2) the death of the organism--the "threshold content" (Vinberg, 1956; Privol'nev, 1954; Stroganov, 1956; Karpevič, 1955, and others).

It has been established that different species of fish and invertebrates react differently to a deficit of O_2. Some of them may exist a certain time with the complete absence of oxygen in the water (worms, molluscs, and others), while others die when the supply of oxygen is relatively great--about 1-2ml/liter. Many sea fish (chamsa, etc.), crustacea, many molluscs feel a lack of oxygen at 1-2 ml/liter (Karpevič, 1955).

It is important to point out that in individuals who demand per unit of weight a relatively high quantity of oxygen, as a rule, the destruction of respiration goes on at a higher level of concentration. "The higher the level of exchange, the higher the level reached by the critical point given large concentrations of dissolved oxygen" (Vinberg, 1956, p. 67).

For example, the norm of oxygen demand for pelagic fishes (chamsa) is about 0.8-1.0 ml for gram/hour, while

bottom fish (bullheads) is about 0.05-0.15 ml per gram per hour. The critical and threshold points in the former are considerably higher than in the latter. A deficit of O_2 is felt by chamsa at 2-3 ml/liter, and asphyxiation sets in at 1.5 ml/liter, while with bullheads it is correspondingly at 1.0 and 0.5-0.7 ml/liter. The critical and threshold content of oxygen is specific for each species.

The critical and threshold concentration of oxygen depends on the intensity of exchange in the animals, and, possibly, on various factors of the environment (temperature, salinity, etc.), when the latter achieve a critical meaning for a given species. The simultaneous action of two unfavorable factors (for example, temperature and salinity) may move the critical point in the direction of a higher concentration of oxygen. For example, the critical concentration of oxygen for the larvae of salmon increases with unfavorable salinity (Karpevič, 1955).

It is very important to consider the physiological condition of the subject animals, since the oxygen threshhold may change in dependence on the stage of their development, age, sexual maturity, and so on.

Consequently, during the study of the effect of lowered oxygen content on the survival of fish and invertebrates

many interesting problems arise. For example:

1) At what concentration of oxygen do the individuals of the studied species begin to feel its lack (the determination of the critical content of O_2).

2) At what concentration of oxygen do individuals die (the determination of the threshold content of oxygen).

3) How long may the subject exist at the critical concentration of oxygen, if that is kept constant.

4)Does the critical point and threshold remain constant for a given subject with the change in a single factor (temperature and salinity, or pH) of the environment, and with a combination change within the favorable and sublethal range for a given species.

5) The effect of the composition of salts and their concentration on the critical point and threshold of the subject.

The Composition of Dissolved Oxygen in Natural Waters

The content of dissolved oxygen in natural waters changes in dependence on the temperature, salinity, depth, atmospheric pressure, and also on the biomasses of phyto- and zooplankton and other aquatic vetetable and animal organisms, and so on. The higher the temperature and salinity of the water, the greater the depth of oceanic waters, the less dissolved oxygen in it. (For the deep waters of the ocean exceptions to this rule are possible.)

The oxygen content dissolved in fresh water at 760 mm of pressure and at differing temperatures is given (in ml/liter) in the oceanographic tables of N.N. Zubov (1931), and in the book of S. M. Dračev, A. S. Razumov, and S. V. Bruevič, et al. (1953).

Here we introduce those tables in a abbreviated form (table 9), but expressed in mg and in ml per liter.

Table 9

Key: 1-Temperature

2-Content of O_2

1 Температура	2 Содержание растворенного кислорода	
	в мг/л	в мл/л
0	14,70	10,29
5	12,89	9,03
10	11,47	8,02
15	10,33	7,22
20	9,39	6,57
25	8,62	6,04
30	7,98	5,57

In the oceanographic tables of Zubov is given a table of dissolved oxygen at various temperatures and salinity (according to Fox).

Below we shall introduce it in an abbreviated form (Table 10).

The quantity of dissolved oxygen in water, shown in the tables, is normal and is taken for 100%.

The Preparation of Water Without Oxygen

In the determination of the oxygen threshold in subject

animals and the moment of their suffocation deoxygenated water is needed, or water with a lowered oxygen content. Such water may be prepared by various methods:

1) The injection of a stream of hydrogen through a balloon with water. In the course of three hours a constant stream of hydrogen lowers the oxygen content of water to 0.19 ml/liter (Bazjakina, 1923; Bodiš, 1945).

2) The injection of CO_2 from a bomb (Veselovskij, 1948).

3) The injection of hydrogen sulphide from Kipp's apparatus (Karpevič, 1940) may give completely deoxygenated water, but with traces of hydrogen sulphide and even with a considerable content of this gas.

4) Boiling with consequent uniting of a flask with boiled and cooled water to a balloon of CO_2 (Dračev, Razumov, and others, 1953).

However, we do not recommend the boiling sea water, or the heating of it to higher than 50-60°, since the loss of certain salts is possible, which destroys the biological and physiological characteristics of the water.

5) The injection of nitrogen from a bomb at first through a hour glass which holds back the oxygen, and then through the water. The water looses oxygen and remains completely harmless for aquatic organisms (the method is used by N.S. Stroganov at the chair of hydrobiology at MGU).

Table 10

The Content of Oxygen Dissolved in Water (in ml/l) at Various Temperatures and Salinities, According to Fox

Таблица 10

Содержание растворенного в воде кислорода (в *мл/л*) при различной температуре и солености, по Фоксу

Температура (в °C)	0‰	2‰	4‰	6‰	8‰	10‰	12‰	14‰	16‰	18‰	20‰	22‰	24‰	26‰	28‰	30‰
0	10,29	10,16	10,04	9,91	9,78	9,65	9,52	9,39	9,27	9,13	9,00	8,88	8,75	8,62	8,49	8,36
2	9,75	9,63	9,51	9,39	9,27	9,15	9,03	8,91	8,80	8,67	8,55	8,43	8,31	8,19	8,07	7,95
4	9,26	9,15	9,04	8,92	8,81	8,70	8,59	8,48	8,37	8,25	8,13	8,02	7,91	7,80	7,69	7,57
6	8,81	8,70	8,60	8,49	8,39	8,28	8,18	8,07	7,97	7,86	7,75	7,65	7,54	7,44	7,34	7,32
8	8,40	8,30	8,20	8,10	8,00	7,90	7,80	7,70	7,60	7,51	7,41	7,31	7,21	7,11	7,01	6,92
10	8,02	7,93	7,83	7,75	7,65	7,56	7,47	7,38	7,28	7,19	7,09	7,01	6,91	6,82	6,73	6,63
12	7,68	7,59	7,50	7,42	7,33	7,25	7,16	7,07	6,98	6,89	6,81	6,73	6,64	6,55	6,46	6,38
14	7,37	7,28	7,20	7,12	7,04	6,96	6,87	6,79	6,71	6,63	6,55	6,47	6,39	6,31	6,22	6,14
16	7,08	7,00	6,93	6,85	6,77	6,69	6,62	6,54	6,46	6,38	6,31	6,23	6,15	6,07	5,99	5,92
18	6,81	6,74	6,67	6,60	6,52	6,45	6,37	6,30	6,23	6,15	6,09	6,01	5,94	5,81	5,79	5,72
20	6,57	6,50	6,43	6,37	6,30	6,22	6,15	6,09	6,01	5,95	5,88	5,81	5,74	5,67	5,59	5,52
22	6,35	6,28	6,22	6,15	6,08	6,01	5,94	5,88	5,81	5,75	5,68	5,61	5,54	5,48	5,41	5,34
24	6,14	6,07	6,01	5,95	5,88	5,81	5,75	5,69	5,62	5,55	5,49	5,43	5,36	5,30	5,23	5,16
26	5,94	5,88	5,81	5,75	5,69	5,63	5,57	5,51	5,44	5,37	5,31	5,25	5,18	5,12	5,06	4,99
28	5,75	5,69	5,63	5,57	5,51	5,45	5,39	5,33	5,26	5,19	5,13	5,07	5,01	4,95	4,89	4,82
30	5,57	5,51	5,45	5,39	5,33	5,27	5,21	5,15	5,08	5,01	4,95	4,89	4,83	4,77	4,71	4,65

6) The sinking into a darkened, completely filled vessel of aquatic plants. In the course of several hours (12-24) the aquatic vegetation in the dark uses for its own respiration all of the dissolved oxygen, but somewhat increases the concentration of CO_2 and lessens the pH. If the bottle of water is not covered a part of the CO_2 goes into the atmosphere, and the water preserves all its biological and physio-chemical properties (this method we have used with success at the advice of G.K. Gorbunov). However, it is necessary to remember that certain plants exude poisonous substances into the water.

7) Almost deoxygenated or with a lowered content of oxygen remains after respiration experiments. In such water there is always CO_2 and the products of exchange, but if the latter are little (the water does not turn blue when titrated with hyposulphate), the water may be used for experiments, all the more so since water in water basins, where with a deficit of O_2 there is often a higher quantity of CO_2, and the products of organic decay, and other substances which absorb oxygen and are harmful to the gas exchange of aquatic organisms. In semi-field conditions it is most convenient of all to use the last two methods for the production of deoxygenated water.

Several authors (Svirenko, 1937; Bazu, 1952, et al.,

have specially studied the effect of various concentrations of carbon dioxide on the young of fresh water fish with varying concentrations of oxygen in closed and open tanks.

Bazu came to the conclusion that with a minimal concentration of oxygen--0.5-1 p.p.m. (Bazu, 1952) which is a threshold for fish. An insignificant quantity of CO_2 equal to 2.5-5 p.p.m. is lethal for the young of the indian carp. But with a raised content of oxygen to 2 p.p.m. and higher even to 100 p.p.m. of CO_2 is not deadly to them.

For the young of the Indian carp, with a length of 1.5-4 cm, which live with a threshold content of oxygen of 0.5- 1 p.p.m., a partial pressure of CO_2 equal to 10-15 p.p.m. is the limit. With even the smallest increase in the content of oxygen (1.0-1.5 p.p.m.) the critical concentration of CO_2 sharply rises. Only a concentration of CO_2 equal to 175-180 p.p.m. becomes dangerous for these young. They survive even 225-250 p.p.m. only in the presence of 2 p. p. m. of oxygen.

The Author recommends the holding of the oxygen pressure in vessels at the time of the transfer of larvae and fingerlings not lower than 2 p.p.m. (2 mg for 1 kg of water); then the rise in the partial pressure of CO_2 will not be dangerous.

In closed vessels CO_2 builds up more than in open ones.

For example, if one puts 16 small carp in an open vessel in 1 liter of water, then in the course of 120 hours not more than 40 p.p.m. are built up, while in closed vessels the CO_2 pressure rises to 220 p.p.m. Therefore, to get deoxygenated water with the least amount of CO_2 one needs a vessel filled with water and plants, kept open, but in complete darkness.

Glassware and Apparatus

For the determination of the oxygen threshold one may use various apparatus and methods, just as in the determination of the "norms" of oxygen demand: 1) in flowing water one may use Krog's apparatus and its modifications (in them one uses flowing water with differing oxygen content); 2) manometer apparatus (Warburg's, etc.); 3) the method of closed vessels and others. Each of these has its advantages and drawbacks (see Vinberg, 1956).

Depending on the goals one chooses the method of study. We cannot describe all the apparatus and methods, and shall dwell only on the most simple which help to determine the oxygen threshold in waters of various salinity under semi-field conditions.

1) The method of closed vessels. The vessels are the same kind as used in the determination of the intensity of respiration, but in work with sea water of differing

salinity one usually uses a series of vessels with an identical volume and form.

2) Apparatus with a movable plug, from which it is possible to at any time take a sample for oxygen.

This piece of equipment is prepared in the following way.

A cylinder of the proper dimensions is selected, of good, transparent glass with completely vertical walls. For example, for molluscs it is desirable to have a relatively wide cylinder of a height of 20-25 cm and with a diameter of 12-15 cm; for pelagian crustacea and for the young of fish one may use a higher and narrower cylinder (25 by10-12 cm, 30 by15-18 cm, and so on).

The vessel must be calibrated, with a scale on its surface which shows the change in the volume of the vessel of 5-10 ml, with the zero on the upper part of the cylinder. For this cylinder a special paraphin plug is made, for which the cylinder is lightly heated and filled with warm water up to the zero mark. The temperature of the water should be close to the temperature of the melting point of the substance selected for the plug, but not higher than 50°, in order that the thick walled vessel does not crack.

Into the water is put a strictly vertical thermometer and a glass pipe, the lower end of which is bent in the

form of a ring. This pipe is simultaneously a siphon and a stirrer, and hits the bottom of the cylinder, while the thermometer goes only to the middle. At the upper end of the siphon is attached a long rubber tube of a diameter of 3-5 mm and with a clamp on the free end. The siphon and thermometer are fixed to a stand.

Then on the surface of the warmed water which fills the cylinder one carefully pours the melted (but not burned) paraphin in a quantity sufficient for the formation of a plug, corresponding to the diameter of the vessel and in a thickness of 3-4cm.

After the cooling of the plug the thermometer and siphon are fixed in. If the paraphin sticks to the glass, one must carefully free it from the sides of the vessel with a scalpel. A slight turn of the siphon will free it from the plug, while the thermometer remains fixed.

With the help of the siphon one may remove the plug from the glass and, after putting in the subject animals, may again be replaced on the surface of the water.

From such a piece of equipment one may at any time take a sample of water for the determination of the oxygen content. It is necessary to check the water level, and calculate the oxygen content on the basis of the actual level.

The lack of full geometric shape is compensated for by the placing of the animals in it in groups. The demand for

oxygen in such a case is quicker than its leaking in through cracks in the plug.

Table 11

The Determination of the Oxygen Threshold for (Name of Animal) in Caspian or Some Other Water of Varying Salinity at a Temperature of 18°

2	3	4	5	6	1 Начало опыта					
№ сосуда	Количество животных	Вес (в г)	S воды (в ‰)	Объем респиратора (в мл)	7 Дата	8 Час	9 Температура	10 Объем пикнометра (в мл)	11 Количество гипосульфита (в мл)	12 Содержание кислорода: в сосуде (13: в мл)	в 1 л: в мл	в 1 л: в %

14 Конец опыта								17 Состояние животных		20 Критическое содержание кислорода (в мл/л или в мг/г)	21 Пороговое содержание кислорода (в мл/л или в мг/г)
15 Дата	16 Час	9 Температура	10 Объем пикнометра (в мл)	11 Количество гипосульфита (в мл)	12 Содержание кислорода: в сосуде (13: в мл)	в 1 л: в мл	в 1 л: в %	18 Количество животных	19 % живых		

Key: 1-Beginning of experiment
2-No. of vessel
3-Quantity of animals
4-Weight (in g)
5-Salinity of water (in ‰)
6-Volume of respirator (in ml)
7-Data
8-Hour
9-Temperature
10-Volume of hour glass
11-Quantity of hyposulphate (in ml)
12-Oxygen content
13-In the vessel
14-End of experiment
15-Data
16-hour
17-Condition of animals
18-Number of animals
19-% of live animals
20-Critical content of O_2 (in ml/liter or mg/g)
21-Threshold content of O_2 (as in 20)

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The Set-up of Experiments

The Determination of the Oxygen Threshold and the Critical Concentration of O_2 for Fish and Invertebrates

In the determination of the oxygen threshold it is simplest of all to use the method of closed vessels.

In a series of respirators (4-6 jars with ground-in stoppers) or aquaria with glass covers are put a group of selected subject animals consisting, as far as possible, of an equal number of individuals, near in size, weight, and physiological condition. Then the jars are filled with water of the same salinity with a normal or lessened content of oxygen, covered with stoppers, and kept under the same conditions of temperature and illumination. After the desired amount of time the jars are opened and the O_2 content is determined, and, simultaneously, the behaviour of the animals is observed.

The weight of the subject animals should not be too high, otherwise the individuals will experience crowding, and there will be a quick dissappearance of O_2 in the jar, and a quick build-up of CO_2 and waste products.

In a vessel with a volume of 1 liter one may place about 0.5-1 g of fish larvae, 0.5- 1g of crustacea, and 5-10 g of molluscs, etc.

However, one must consider that pre-experimental weighing is possible only with hardy organisms (molluscs, crabs, worms, young and adult fish, etc.). Many forms do not withstand

weighing, and, therefore, the weight of the animals before an experiment is determined by the determination of the approximate average weight of an individual, and, more exactly, after the experiment.

Knowing the "norm" of the respiration of the subject animals at a given temperature, salinity, and weight, and, likewise, the original oxygen content contained in the vessel, one may determine beforehand the time during which the normal respiration of the animals is possible, and the time when the oxygen content falls below 50% of normal. After this one must make more frequent observations on the behaviour of the animals in the jars.

The arival of the critical content of oxygen is determined by the increased motion of the legs in crustacea, of the gills of fish, and by the unusual extension of the siphon in molluscs, and so on.

The arival of the threshold content of O_2 is determined at the moment of the deaths of 30-50% of the subjects.

Depending on its purpose, the respirator is opened either at the moment of the first agitation of the animals, or at their death. In the first case the content of O_2 in the water is critical, in the second it is the threshold content.

The determination of oxygen is carried out, as usual, by Winkler's method, but in the final calculation one introduces the indicator of the % of oxygen saturation in the water (see Table 11).

If it is necessary to determine the moment of suffocation in animals in waters of varying salinity, then the jars are filled with the selected water, and the observations on the animals are carried out as described above. One must only remember that in water, the salt content of which is sublethal for a given species, the oxygen threshold is raised. An example of notation is given in table 11.

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Addemdum

List of equipment and reagents necessary for the conduct of experiments on the survival of organisms in waters of varying salinity.

Name Наименование	Dimensions and volume Размер (объем)	Quantity Количество
Aquaria (glass, plexiglass		items
Аквариумы (стеклянные, из плексигласа, эмалированные и т. д.) enamel, etc	from 5 liters от 5 л	по 10—15 шт. each size каждого размера
Банки материальные jars	various Разного размера sizes	20—30 шт. items,
Бутыли—водохранилища bottles	2—15 л	по 10 шт. etc.
Бутыли для доставки воды из моря sample bottles	15—25 л	10—20 шт.
Бутыль для азотнокислого серебра bottle for	1—2 л	1 шт.
Бюретки burettes	2—5 мл	по 2 шт.
Воронки pipes	various sizes Разного размера	10 шт.
Зажимы Мора и Гофмана clamps	Разные various	50 шт.
Кристаллизаторы crystallizers	15—50 см в диаметре	10—15 шт.
Кюветы для выпаривания морской воды cuvettes	more than 2 lit. Свыше 2 л	10 шт.
Кружки фарфоровые flasks	250—1000 мл	5 шт.
Колбы мерные graduates	100—1000 мл	5 шт.
Колбы Эрленмейера Erlenmeyer	100—1000 мл	10—20 шт.
Мензурки graduates flasks	10—500 мл	10 шт.
Пипетки биологические pipettes	Разного размера	5—10 шт.
Пипетки химические, градуированные и неградуированные ditto glass		10—20 шт
Палочки стеклянные rods	1—50	10 шт.
Пробирки биологические test tubes		1000 шт.
Стаканы Бунзена Bunsen jars	Разного размера 250—500 мл	10—15 шт.
Стаканы химические glasses	1—5 л	по 10 шт.
Солонки salt cellars	Разного размера	10 шт.
Скляники для реактивов glasses	" "	10 шт.
Сачки из шелкового газа	" "	10 шт.
Трубки стеклянные для сифонов glass pipe	Разные	10 шт.
Трубки каучуковые rubber pipe	2—10 мм в диаметре	около 5 кг с. 5 kg.
Тигелек для титрования хлора воды crucible for titration of chlorine	Разного диаметра various diameters	" 5 кг
Цилиндры мерные graduates	10—15 мл	2 шт.
Чашки Петри Petri dishes	100—2000 мл	5 шт.
Чашки Коха " "	—	10—15 шт.
Шпатели Sputulas		10—15 шт.
Часовые стекла watch glasses	Разные various	1—2 шт.
Пробки резиновые и корковые corks	Разные various	10 шт.
Стекла-крышки для аквариумов glass covers for aquaria		

List of equipment and reagents necessary for the conduct of experiments on the study of the respiration of aquatic animals in waters of varying salinity

Name Наименование	Dimension Размер	Number Количество (в шт.) of items
Apparatus **Оборудование**		
wide mouth jars with stoppers Банки широкогорлые с притертыми пробками—респираторы	100—250 *мл* 500—2000 *мл* 3000—5000 *мл*	15
jar Банки материальные	Разного размера	20—50
bottle Бутыли темного стекла	2—3 *л*	12
burette Бюретки	5— 10 *мл*	2
Воронки стеклянные	Разные various	5
scales Весы пружинные	0,5—2 *г*	2
Кристаллизаторы crystallizers	Разные various	20
cuvette Кюветы		10
Колбы мерные measuring flask	100—2000 *мл*	5
Erlenmayer Эрленмейера flasks	100—500 *мл*	5—10
flat bottom . плоскодонные flask	2000 *мл*	1—2
Капельницы . droppers	25—50 *мл*	2—3
Лупа ручная	2×	1
Натронные трубки . pipe	—	1
graduated pipettes Пипетки градуированные	1—10 *мл*	По 5
biological pipettes . биологические (с грушей)	Разные	5—10
Пикнометры . hom-glass	25—50 *мл*	20—30
Пробирки биологические	Разные	1000
Стаканы Бунзена Bunsen jar	3—5 *л*	25
. химические	Разные	10
O_2 jars Склянки кислородные с притертыми пробками w. stoppers	50—100 *мл*	20—30
Палочки стеклянные glass rods		

Реактивы Reagents		
Гипосульфит ($Na_2S_2O_3$)	250 *г*	grams
Калий двухромовокислый ($K_2Cr_2O_7$)	250 .	
Калий хромовокислый (K_2CrO_4)	100 .	
Калий йодистый (KJ)	500 .	
Калий йодноватокислый (KJO_3)	250 .	
Крахмал картофельный	100 .	
Марганец хлористый ($MnCl_2$)	500 .	
Натронная известь	2000 .	
Натр едкий (NaOH)	2000 .	
Серная кислота (H_2SO_4)	1000 .	
Соляная кислота (HCL)	300 .	
Азотнокислое серебро ($AgNO_3$)	100 .	
Хлористый натрий (NaCl)	100 .	
Нормальная вода normal water	2 баллона	balloons

9*

131